

## 7

## Approaches for Functional Modification or Cross-linking of Chitosan

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### 7.1 Introduction

Why is chemical modification of chitosan needed? The partial answer to this question may be explained by the low hemocompatibility and water-insoluble nature of chitosan. Additionally, modification is required in order to manipulate other properties of chitosan to meet specific needs. Chitosan is a polysaccharide obtained mainly from crustacean shells and is composed of 2-amino-2-deoxy- $\beta$ -D-glucan combined with glycosidic linkages. The primary amine groups render special properties that make chitosan very useful in pharmaceutical applications. Chitosan's nontoxicity, biodegradability, and biocompatibility make it suitable for various biomedical applications such as in drug delivery [1–4], gene delivery [5,6], wound dressing [7–11], and tissue engineering [12,13]. Given the wide and diverse range of potential applications, chitosan may be chemically modified so as to maximize the polymer processability, solubility, the antimicrobial activity, and ability to interact with other substances. Modified chitosan is expected to show different features from those of native chitosan. With regard to drug delivery applications, the new properties of modified derivatives include enhanced solubility in water and thereby better biodistribution or bioavailability when they are administered parenterally. For example, carboxymethylation of chitosan increases the solubility of chitosan at neutral and alkaline pH values without affecting other important characteristics [14–19]. The hemocompatibility may also be increased as the positive charge of the system is reduced.

Important chemical modification methods of chitosan are discussed in this chapter, including carboxymethylation, thiolation, succinylation, grafting, and copolymerization, among others. This chapter also

intends to systematize related issues, including the various methods of chitosan grafting and their formulation for drug delivery [20]. In recent years, much attention has been given to water-soluble, stimuli-responsive polymeric systems based on chitosan, which show a phase transition in response to external stimuli such as temperature, pH, specific ion concentration, and electric field [21]. Among all the studied stimuli-sensitive materials, temperature- and pH-responsive polymers have drawn the most attention, because these are important physiological factors in the body, and some diseases manifest themselves by changes in either temperature or pH, or even both [21]. In particular, several research groups have reported on the preparation of pH- and temperature-sensitive polymers based on poly(*N*-isopropylacrylamide) (PNIPAAm) and poly(*N*-vinylcaprolactam) (PNVCL) for biomedical applications, where PNVCL showed a better defined response toward temperature than PNIPAAm [3,22]. Thus, in the current chapter, an overview on the drug delivery applications of cross-linked [23] and chemically modified chitosan is given. Important modified and cross-linked derivatives include carboxymethyl-chitosan (CMC), *O*-carboxymethyl-chitosan (*O*-CMC), *N,O*-carboxymethyl-chitosan (*N,O*-CMC) and *N*-carboxymethyl-chitosan (*N*-CMC) [14–19], succinyl-chitosan [24–32], thiolated chitosan [33–43], and chitosan grafted with PNIPAAm and PNVCL (chitosan-*g*-PNIPAAm and chitosan-*g*-PNVCL) [44–47].

## 7.2 General Awareness of Chitosan Cross-Linking Methods

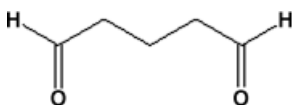
### 7.2.1 Chemical Cross-Linking

Cross-linking happens when a chemical or compound, referred to as the “cross-linker,” makes intermolecular covalent bridges between the polymer chains [23]. Chemical cross-linkers include glutaraldehyde, genipin, glyoxal, dextran sulfate, 1,1,3,3-tetramethoxypropane, oxidized cyclodextrins, ethylene glycoldiglyceryl ether, ethylene glycol diglycidyl ether (EGDE), and diisocyanate, among others [23]. Some of the commonly used cross-linking agents will be discussed below.

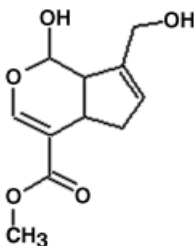
#### 7.2.1.1 Glutaraldehyde

Glutaraldehyde (Figure 7.1) can easily cross-link with chitosan, because of its active aldehyde groups. The mechanism involves the formation of Schiff’s base via nucleophilic attack by the nitrogen of the amino group (from chitosan) on the carbon of the glutaraldehyde, which displaces the oxygen of the aldehyde resulting in the C = N bond [23]. However, there are concerns with the use of glutaraldehyde as it is suspected to impart toxicity, which may result in the decline of biocompatibility of systems [48].

Several studies have reported on the use of glutaraldehyde as a cross-linker for chitosan-based materials. For instance, glutaraldehyde cross-linked chitosan–poly(vinyl alcohol) (PVA) hydrogels were developed as injectable drug delivery systems [49]. Also, pH-responsive, freeze-dried chitosan–polyvinyl pyrrolidone (PVP) hydrogels [50] and chitosan–PVA hydrogels [51] were developed for drug delivery applications by cross-linking with glutaraldehyde. The potential of post cross-linking of chitosan, after preparing a semi-interpenetrating polymer network (semi-IPN) with PNIPAAm to create temperature-responsive and pH-sensitive IPNs for drug delivery, has also been studied [44]. Further, CMC [52] and *N*-(2-carboxybenzyl)-chitosan hydrogels [53] have been prepared by reacting glutaraldehyde with the respective chitosan derivative.



**Figure 7.1** Chemical structure of glutaraldehyde.



**Figure 7.2** Chemical structure of genipin.

Hydrogels of poly(ethylene glycol) (PEG)-grafted-chitosan cross-linked with glutaraldehyde were developed for drug delivery [51]. Poly(*N*-acryloylglycine-chitosan) hydrogels were also developed by irradiating the solution of *N*-acryloylglycine mixed with chitosan in the presence of glutaraldehyde as a cross-linker and 2,2-dimethoxy-2-phenyl acetophenone as a photo-initiator [54].

Glutaraldehyde cross-linked chitosan beads for drug delivery were obtained by extruding chitosan–PEG [55], chitosan–glycine [56], chitosan–alanine [57], and chitosan–PVP [58] solutions as droplets into a sodium hydroxide–methanol solution. Resulting beads were washed with water and cross-linked with glutaraldehyde. In another work, glutaraldehyde cross-linked chitosan-based beads were developed in a simple way, by using a chitosan solution containing glutaraldehyde to form beads in sodium hydroxide solution [59]. Semi-IPN microspheres of acrylamide-*g*-chitosan were developed by adding solutions of acrylamide-*g*-chitosan to paraffin [60]. The required amount of glutaraldehyde was added to the resulting emulsion under stirring in order to cross-link the microspheres.

#### 7.2.1.2 Genipin

One of the relatively new cross-linking agents is the naturally occurring substance genipin (Figure 7.2). It is an excellent cross-linker for polymers containing amino groups and forms a blue gel upon spontaneous reaction with amino groups [61]. As a result, genipin cross-linked chitosan hydrogels have a bluish appearance. The cross-linking mechanism involves a nucleophilic attack by the amino group of chitosan on to the olefinic carbon atom at C-3 of genipin, followed by the opening of the dihydropyran ring. The formation of a secondary amide and a heterocyclic amino linkage leads to the cross-linking of chitosan [62].

Different examples of the use of genipin as a cross-linker of chitosan have been reported. For example, genipin was used for cross-linking chitosan and chitosan–poly(ethylene oxide) by mixing the corresponding polymer solutions with genipin [62]. Genipin cross-linked chitosan microspheres were also prepared by other techniques like spray drying [63] or water-in-oil emulsion [64,65]. Hydrogels of *O*-CMC–alginate were developed by cross-linking with genipin for protein drug delivery [64]. In another example, chitosan–alginate beads were developed by dropping chitosan solution into a gelling bath containing a mixture of alginate and genipin [66].

#### 7.2.1.3 Glyoxal

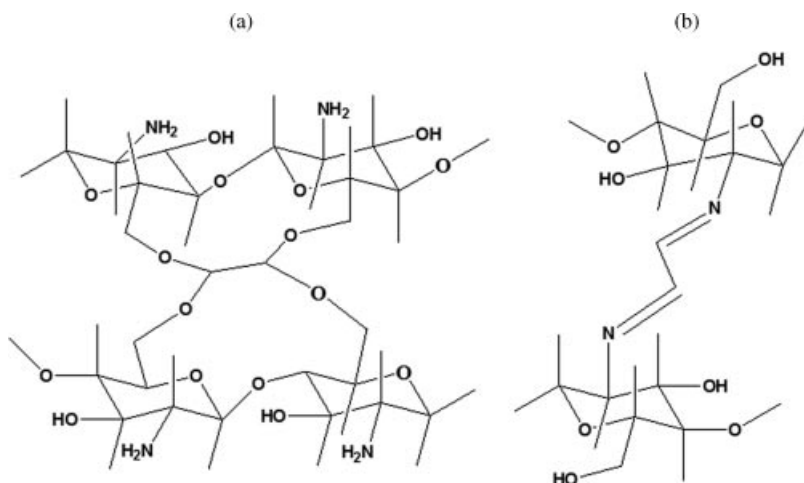
Glyoxal (Figure 7.3) can cross-link chitosan in the same way as glutaraldehyde (Figure 7.4). Selected examples of its use as a cross-linker of chitosan include the preparation of enantioselective L-aspartic acid-imprinted chitosan [67] and superporous chitosan hydrogels [68].

#### 7.2.1.4 Dextran Sulfate

Dextran sulfate is a biocompatible polyanionic polymer. It is a highly branched polysaccharide (Figure 7.5) with 1–6 and 1–4 glycosidic linkages, with approximately 2.3 sulfate groups per glucosyl unit. It is widely used in medical field as a plasma volume expander. Several preparations of dextran sulfate have shown promising



**Figure 7.3** Chemical structure of glyoxal.

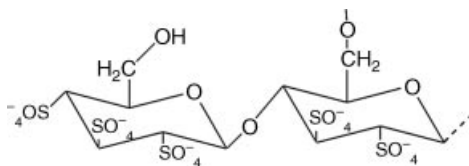


**Figure 7.4** A description of the mechanism for glyoxal cross-linking of chitosan. Glyoxal reacts with hydroxyl groups (a) and amino groups (b) in chitosan. Redrawn from reference [67].

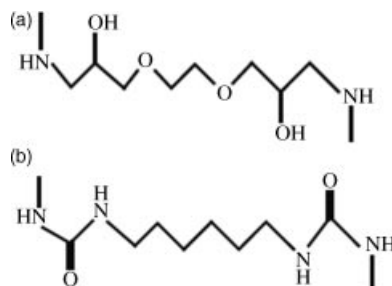
anticoagulant [69] and fibrinolytic [70] activity. Dextran sulfate can also be used as a cross-linker for chitosan. For instance, chitosan–dextran sulfate micro- and nanoparticles were formed by the electrostatic interaction between the protonated amino groups of chitosan and the sulfate groups of dextran sulfate. These particles were used for controlled drug delivery applications since the synthesis route is simple and can be done in mild conditions [71,72]. The surface charge of this type of particles were tunable by varying the ratio of the two-polymer concentrations [73]; also, prepared particles have good stability and do not need any stabilization or additional cross-linking agent. Chitosan–dextran sulfate nanoparticles have been reported for the oral delivery of insulin [74], intravenous delivery of antiangiogenic peptides [75], and controlled delivery of low-molecular weight (MW) drugs [76].

#### 7.2.1.5 Bifunctional Cross-linking Agents

In addition to glutaraldehyde and genipin, numerous bifunctional reagents have been used to cross-link chitosan covalently, such as epichlorohydrin, diisocyanate (Figure 7.6), or epoxy compounds, 4-butanediol diglycidyl ether or ethylene glycol diglycidyl ether (EGDE; Figure 7.6) [77]. Among those bifunctional



**Figure 7.5** Chemical structure of dextran sulfate.



**Figure 7.6** Structure of ethylene glycol diglycidylether (EGDE) (a) and of diisocyanate (b).

reagents, EGDE may be the most suitable cross-linker for reaction with chitosan to prepare flexible films. This is based on the observation that while cross-linking with glutaraldehyde increased the tensile strength and decreased the elongation of 6-*O*-CMC–water-soluble polyurethane (WPU) composite membranes, the elongation of 6-*O*-CMC–WPU membranes increased upon reaction with EGDE [78]. Recently, a novel biodegradable stent made of chitosan–poly(ethylene oxide) blended films cross-linked with EGDE, which exhibited shape memory characteristics, was developed for the sustained release of sirolimus [79].

## 7.2.2 Radiation Cross-Linking

Radiation cross-linking does not require heat or a catalyst; thus, no additional toxic chemical is introduced into the system. Radiation polymerization has been utilized by researchers to obtain IPNs for drug delivery applications [80–83]. Also, photo-cross-linkable chitosan was developed by introducing azide and lactose moieties on chitosan through a condensation reaction [84], and these hydrogels found applications in the release of fibroblast growth factors and heparin [85].

## 7.2.3 Physical Cross-Linking

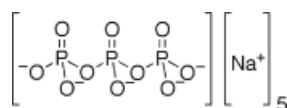
In contrast to covalent bonding of chemical cross-linking, physical cross-linking is obtained by using cross-linkers that establish ionic interactions between the polymer chains. Two well-recognized examples of physical cross-linkers of chitosan are pentasodium tripolyphosphate (TPP) and calcium chloride.

### 7.2.3.1 Pentasodium Tripolyphosphate

TPP (Figure 7.7) is a well-known cross-linking agent for the preparation of micro- and nanoparticles of chitosan and its derivatives [18,45,86,87]. For instance, 5-fluorouracil-loaded chitosan-g-PNVCL nanoparticles [88], gliclazide-loaded chitosan microparticles [89], rifampicin- and hydroxyurea-loaded chitosan microspheres [90], and exotoxin–chitosan microparticles obtained by spray drying were developed using TPP as a cross-linker [91].

### 7.2.3.2 Calcium Chloride

This is a well-known physical cross-linker for a variety of materials having one or more active carboxyl functionalities, including alginate, *O*-CMC, and *N,O*-CMC, among others. One of the actual interests in



**Figure 7.7** Chemical structure of pentasodium tripolyphosphate (TPP).

calcium chloride, among others, is connected with its chelating ability with minimal or negligible toxicity [18]. The formulation of nanosystems of polysaccharide derivatives using calcium chloride will be discussed later in this chapter.

### 7.3 Modified Chitosan: Synthesis and Characterization

#### 7.3.1 Synthesis of Water-soluble Chitosan Derivatives

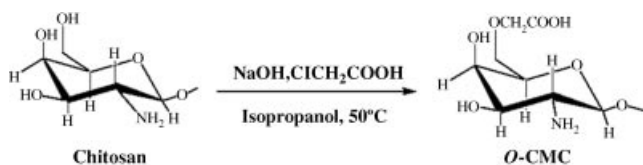
##### 7.3.1.1 Carboxymethylation

One of the most important chemical modification methods of chitosan is carboxymethylation. Carboxymethyl derivatives of chitosan (CMC) were found to be nontoxic, anionic, and water soluble. Because of these excellent properties, CMC found applications in biomedical and environmental fields [14,92–95]. Depending on the position of the carboxymethyl substitution, these derivatives can be classified as *O*-CMC, *N*-CMC, and *N,O*-CMC [14,93].

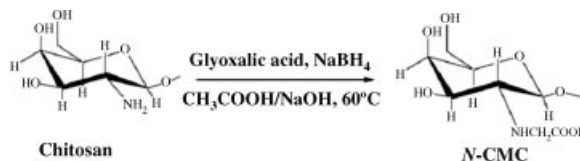
The synthesis protocol for *O*-CMC is well described [14,16,17], and it involves the carboxymethylation reaction of chitosan powder with monochloroacetic acid using isopropyl alcohol as the solvent system. The reaction procedure involves the treatment of chitosan with 50% sodium hydroxide solution at 18°C for 12 h followed by the reaction with chloroacetic acid. Depending on the experimental conditions, such as the reaction temperature, carboxymethyl derivatives with different degrees of substitution may be obtained [14]. The reaction scheme for the synthesis of *O*-CMC from chitosan is depicted in Figure 7.8.

The synthesis protocol for *N*-CMC is also well described [14,96]. It involves the formation of an aldimine by the reaction of the free amino groups of chitosan with glyoxylic acid, followed by the reduction of the aldimine product by sodium cyanoborohydride [14,96]. The reaction scheme is depicted in Figure 7.9.

A number of reports on the synthesis of *N,O*-CMC from chitosan is available [14,19,97]. The synthesis involves the substitution by carboxymethyl groups of some of the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure. It involves the carboxymethylation of chitosan using monochloroacetic acid in alkaline medium (Figure 7.10). *N,O*-CMC is hydrophilic and a typical kind of amphoteric polyelectrolyte with antibacterial effect [98]. It is an excellent candidate for the preparation of membrane



**Figure 7.8** Reaction scheme showing the synthesis of *O*-CMC from chitosan. Adapted from [14], Copyright (2010), with permission from Elsevier.



**Figure 7.9** Reaction scheme showing the synthesis of *N*-CMC from chitosan. Adapted from [14], Copyright (2010), with permission from Elsevier.



**Figure 7.10** Reaction scheme showing the synthesis of *N,O*-CMC from chitosan. Adapted from [14], Copyright (2010), with permission from Elsevier.

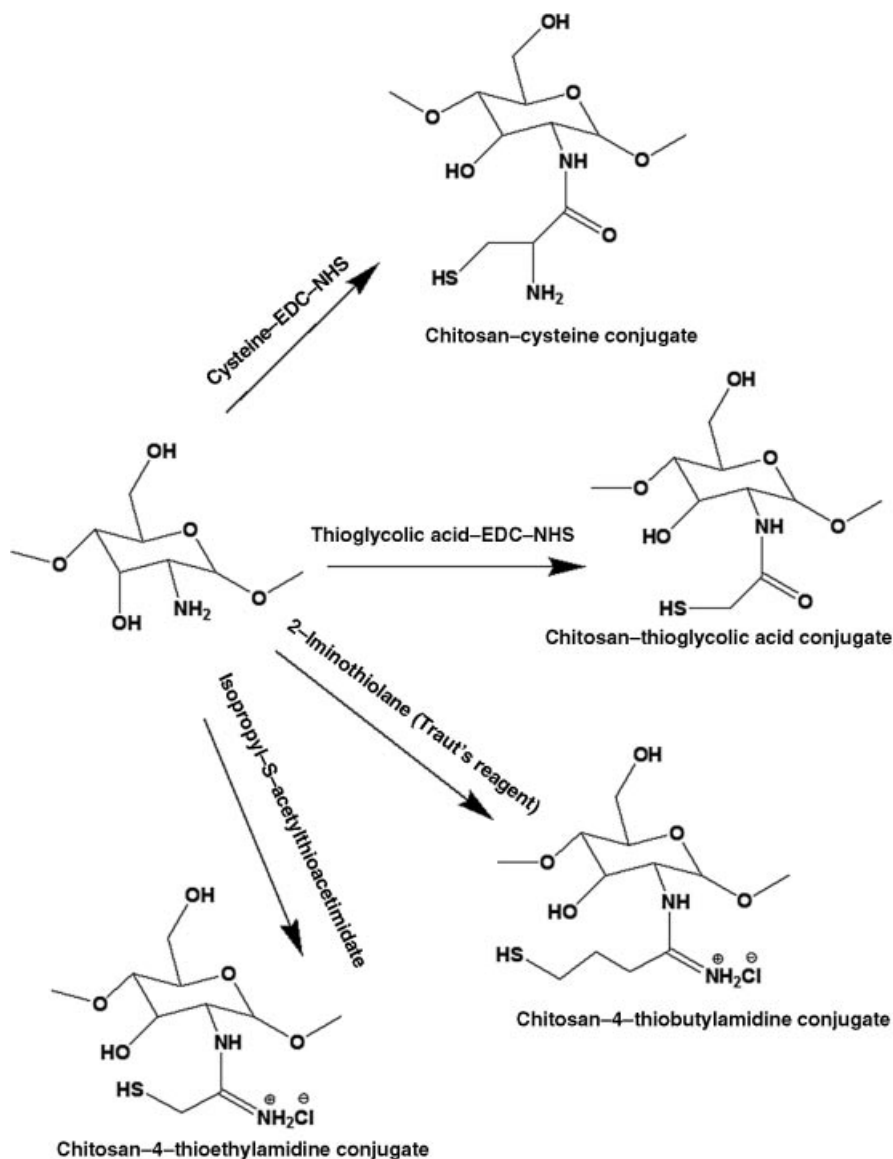
materials, which are used in filtration processes [99]. Nanoparticles of *O*-CMC [2,4,18] and *N,O*-CMC [18,19] have been prepared via the cross-linking reaction with  $\text{CaCl}_2$  and TPP, respectively.

### 7.3.2 Thiolation

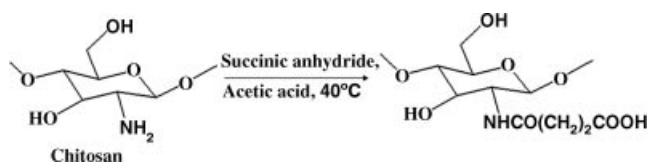
Thiolated chitosan is obtained by the substitution with thiol-bearing moieties of the chitosan backbone (position 2 of the glucosamine subunits of chitosan) via the formation of amide or amidine bonds (Figure 7.11) [34,36,40–42]. Depending upon the agents used for thiolation, different thiolated chitosan derivatives can be obtained. These include chitosan–thioglycolic acid conjugates [41,42], chitosan–cysteine conjugates [36,40], and chitosan–4-thio-butyl-amidine conjugates [34]. In the case of the formation of amide bonds, the carboxylic acid group of the ligands cysteine and thioglycolic acid reacts with the primary amino group of chitosan as mediated by a water-soluble carbodiimide. Thiolation reaction with Traut's reagent (2-iminothiolane) has the advantages of being a one-step reaction and protecting the thiolating agent from oxidation. The degree of thiol substitution in thiolated chitosan can be obtained based on Ellman's method for assaying thiols [33–43]. Thiolated chitosan possesses better mucoadhesiveness and permeation properties as compared to unmodified chitosan [33–43]. The improved mucoadhesion of thiolated chitosan can be explained based on the fact that there is the possibility of formation of covalent bonds between thiol groups of the polymer and cysteine-rich subdomains of glycoproteins in the mucus layer. These covalent bonds were reported to be stronger than noncovalent bonds, such as the ionic interactions established between chitosan and the anionic substructures of the mucus layer. Nanoparticles of thiolated chitosan may be obtained as a result of an ionic cross-linking reaction of thiolated chitosan with TPP [100]. Thiolated chitosan nanoparticles have been studied for applications in drug delivery as well as for permeation enhancement [33–43].

### 7.3.3 Succinylation

The general reaction for the obtention of *N*-succinyl–chitosan is showed in Figure 7.12. One of the important succinyl–chitosans, *N*-succinyl-*N'*-octyl-chitosan [32], which can form micelles in an aqueous media, has been prepared by modifying the amino group with a hydrophobic long-chain alkyl functionality and a hydrophilic succinyl moiety [47]. An amphiphilic derivative of succinyl–chitosan has also been reported [29]. The results showed that the modified chitosan ((2-hydroxypropyl-3-butoxy)-propyl-succinyl-chitosan) can concentrate on the surface of water to decrease the surface tension and can associate with hydrophobic chains to form aggregates in the solution. The abilities to decrease the surface tension and to form aggregates were promoted by increasing the degree of substitution of the hydrophobic group and the addition of salt [29]. Synthesis and evaluation of *N*-succinyl–chitosan nanoparticles toward local hydroxycamptothecin delivery have also been reported [32]. The synthesized *N*-succinyl–chitosan derivative, which could self-aggregate to form nanoparticles in distilled water, found potential application for hydrophobic anticancer drug delivery.



**Figure 7.11** Different thiol functionalization strategies for chitosan.



**Figure 7.12** Reaction scheme for the synthesis of succinyl-chitosan from chitosan.



### 7.3.4 Chitosan-Grafted Polymers

According to the International Union of Pure and Applied Chemistry (IUPAC), grafting in polymer chemistry refers to the reaction in which one or more species of blocks are connected to the chain of a macromolecule as side chains, having constitutional or configurational features that differ from those in the main chain. In general, grafting can improve the properties of materials by controlling various parameters, namely, the comonomer ratio, solvent concentration, and temperature, among others. Depending on the requirement, novel properties such as enhanced water solubility, lower critical solution temperature (LCST), improved drug-loading capacity, and hemocompatibility can be achieved. Grafting of chitosan with different functionalities may improve the biomedical applications especially in drug delivery [101–106]. Some of the grafting techniques for chitosan are discussed in the following subsections.

#### 7.3.4.1 Grafting Initiated by Free Radicals

In recent years, a number of initiators, such as ammonium persulfate (APS), potassium persulfate (PPS, or  $K_2S_2O_8$ ), ceric ammonium nitrate (CAN), thiocarbonate–potassium bromate (TCPB), potassium diperiodatocuprate (III) (PDC), 2,2′-azobisisobutyronitrile (AIBN), and ferrous ammonium sulfate (FAS), have been developed for grafting copolymerization [104,105]. For example, using PPS and sodium bisulfite ( $NaHSO_3$ ) as redox initiators, 4-vinylpyridine was grafted onto chitosan under homogeneous as well as heterogeneous conditions [104]. In another work, a thermosensitive hydrogel was developed by block copolymerization of monomethoxy-poly(ethylene glycol) onto a chitosan (chitosan–PEG) backbone, using PPS as a free radical initiator [105]. The prepared block copolymer exhibited a thermoreversible transition from an injectable solution at low temperature to a gel at body temperature. The study of the gelation behavior showed the applicability of chitosan–PEG block copolymers in the biomedical field. In addition to the stated examples, several important grafting examples of chitosan are presented in Table 7.1.

#### 7.3.4.2 Radiation-Induced Grafting

In addition to free radical initiators, radiation has also been used to induce the grafting of several natural polymers. In one study, graft copolymerization of butyl acrylate onto chitosan has been performed using  $\gamma$ -irradiation [116]. It was found that the grafting percentage increased when the monomer concentration and total radiation dose increased or when the chitosan concentration and reaction temperature decreased.

**Table 7.1** Free Radical-Initiated Grafting Techniques for Chitosan

Co-monomer or -polymer	Initiators used	References
Poly(4-vinylpyridine)	APS	[106]
Poly(3-hydroxy-butylate)	APS	[107]
Polyaniline	APS	[108]
Vinyl acetate	CAN	[101]
Polyacrylamide	CAN	[102]
Poly(acrylic acid)	CAN	[102,106]
Poly(4-vinylpyridine)	CAN	[106]
<i>N,N</i> -dimethyl- <i>N</i> -methacryloxyethyl- <i>N</i> -(3-sulfopropyl) ammonium	CAN	[109]
Poly(acrylonitrile)	CAN	[110]
2-Hydroxy-ethyl-methacrylate	CAN	[111]
Vinyl pyrrolidone	PPS	[106]
Acrylonitrile	PPS	[112]
2-Acrylamide-2-methyl-propanesulfonic acid	PPS	[114]
Methyl acrylate	PDC	[113]
Vinyl monomers	AIBN	[103,115]

AIBN: 2,2′-azobisisobutyronitrile; APS: ammonium persulfate; CAN: ceric ammonium nitrate; PDC: potassium diperiodatocuprate (III); and PPS: potassium persulfate.

Similar work has also been reported for grafting chitosan with poly(hydroxyethyl methacrylate) (PHEMA) in the presence of UV light [117]. In this case, sulfite oxidase enzyme was then covalently immobilized onto the matrix of the grafted polymer. After the completion of the photo-induced polymerization reaction, *p*-benzoquinone was coupled onto the polymer network for activation of the chitosan–PHEMA copolymer. This study highlighted the feasibility of using chitosan for electrochemical biosensor applications [117]. Microwave irradiation has also been used for grafting chitosan with polyacrylonitrile [118]. The effects of reaction variables, such as monomer or chitosan concentration, microwave power, and exposure time on the graft copolymerization, were studied. Parameters such as solvent composition, monomer concentration, radiation dose rate, and total dose and time were found to affect the rate of grafting and homopolymerization.

#### 7.3.4.3 *Enzymatic Grafting*

Grafting techniques by enzymes allow for a number of advantages in the synthesis of polymers [119]. Enzymes can selectively and specifically eliminate the hazards associated with chemical reagents. Also, they can modify the macromolecular structure, thereby enhancing the polymer function [119–123]. Enzymatic modification of chitosan results in derivatives with unique pH-sensitive, water-soluble, and adhesive properties. For instances, tyrosinase enzyme can effectively graft phenolic compounds onto chitosan, thus conferring water solubility under basic conditions [122]. In slightly acidic media (pH 6), chitosan could be modified under homogeneous conditions with the natural product chlorogenic acid. The modified chitosan was soluble under both acid and basic conditions, even when the degree of modification was low. Since it is possible for quinones to undergo either or both type of reactions with amines, as well as oligomer-forming reactions with other quinones, it is common for reactions between quinones and amines to yield complex mixtures of products [122].

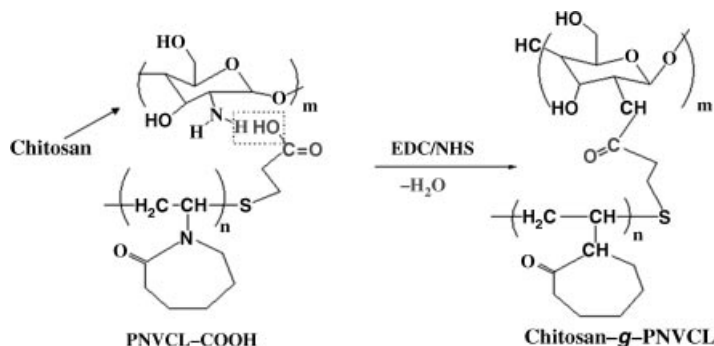
In one report, the feasibility of using tyrosinase as a catalyst for grafting hexyloxyphenol onto chitosan was investigated [123]. The method employed tyrosinase to convert the phenol into a reactive *o*-quinone, which undergoes a subsequent non-enzymatic reaction with chitosan under homogeneous conditions. The heterogeneous modification of a chitosan film was found to produce a hydrophobic surface due to the substituent, while homogeneously modified chitosan exhibited rheological properties characteristic of associating water-soluble polymers. In order to confer functional properties to chitosan, horseradish peroxidase has also been used as a catalyst in grafting reactions [121].

#### 7.3.4.4 *Cationic Graft Polymerization*

The grafting reaction onto chitosan is also performed by using living cationic polymerization. Grafting of chitosan with living poly(isobutylvinyl ether) and poly(2-methyl-2-oxazoline) cations with controlled molecular weight distribution has been reported [124]. In this study, researchers have analyzed the effect of the molecular weight of living polymer cations on the number of grafted polymers; it was found that the number of grafted polymer chains decreased with the increasing molecular weight of living polymer cations.

#### 7.3.4.5 *Chitosan-Grafted Thermosensitive Polymers*

Synthesis and self-assembly of tunable thermosensitive chitosan amphiphilic copolymers have been reported via click chemistry [125]. In this way, chitosan grafted with copolymers of 2-(2-methoxyethoxy)ethyl methacrylate (MEO<sub>2</sub>MA) and oligo(ethylene glycol) methacrylate (OEGMA) (chitosan-*g*-P(MEO<sub>2</sub>MA-*co*-OEGMA)) was synthesized by the “graft onto” method via click chemistry. It was observed that amphiphilic chitosan-*g*-P(MEO<sub>2</sub>MA-*co*-OEGMA) can be assembled into micelles in water. The self-assembling behavior and tunable thermosensitive properties of chitosan copolymer micelles were investigated. The LCST values of micelle solutions were able to be tuned by altering the molar ratio of MEO<sub>2</sub>MA and OEGMA. The micelles could also reversibly swell and shrink in response to external temperature. The obtained thermosensitive amphiphilic graft copolymers have both the unique properties of P(MEO<sub>2</sub>MA-*co*-OEGMA) and chitosan, which can be utilized for thermoresponsive drug delivery in combination with different thermal ablation therapies [125].

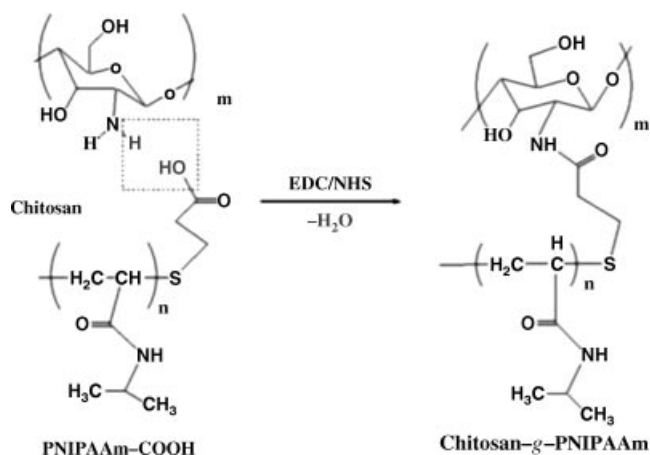


**Figure 7.13** The reaction scheme for the synthesis of chitosan-g-PNVCL using a 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC)-*N*-hydroxysuccinimide (NHS) mediated amidation reaction. Redrawn from reference [45].

A novel magnetic nanoparticle drug carrier for controlled drug release has been reported to respond to changes in external temperature or pH, resulting in longer circulation time and reduced side effects of the delivered drug (doxorubicin) as compared to the native drug [126]. The novel nanocarrier is described as a functionalized magnetite ( $\text{Fe}_3\text{O}_4$ ) core that is conjugated with doxorubicin via an acid-labile hydrazone bond and encapsulated by the thermosensitive smart polymer, chitosan-g-poly(*N*-isopropylacrylamide-co-*N,N*-dimethylacrylamide). The developed smart polymer exhibited a LCST of  $38^\circ\text{C}$ . The drug release was appreciably low below the LCST as opposed to temperatures above the LCST. In each case, there was an initial rapid drug release, followed by a controlled release in a second stage, especially in a mild acidic buffer solution [126].

#### 7.3.4.6 Nanoparticles Produced with Chitosan-Grafted Thermosensitive Polymers

Recently, chitosan-g-PNVCL nanoparticles have been reported as carrier systems for 5-fluorouracil using TPP as a cross-linker [45]. The synthetic route for obtaining the modified chitosan is presented in Figure 7.13. Nanoparticles showed an excellent hemocompatibility after 4 h of incubation with erythrocytes, thus



**Figure 7.14** The reaction scheme for the synthesis of chitosan-g-PNIPAAm using a 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC)-*N*-hydroxysuccinimide (NHS) mediated amidation reaction. Reprinted from [127], Copyright (2011), with permission from Elsevier.

suggesting that intravenous administration of these formulations may be possible. In addition, it has been reported that the modified chitosan may be useful for both hydrophobic [88] and hydrophilic drug encapsulation [45] via a thermosensitive drug release mechanism. Also, nanoparticles produced with chitosan modified with PNIPAAm–COOH (chitosan-g-PNIPAAm) were developed for the delivery of curcumin [127]. The synthetic route for obtaining the modified chitosan is presented in Figure 7.14. TPP was used as a cross-linker to obtain the nanoparticles. Interesting results were observed when using nanoparticles, namely, the specific toxicity of curcumin toward cancer cells [127].

## 7.4 Applications of Modified Chitosan and Its Derivatives in Drug Delivery

The major applications of modified as well as cross-linked chitosan involve the development of the nanoformulations, which can act as improved therapeutic carrier systems for drug delivery. The advantages of modified and cross-linked nanoformulations include high solubility, good loading efficiency, and more sensitivity to release the drugs at different pH values. Several examples are described in this section.

Alginate–folic acid-modified chitosan nanoparticles were developed by a TPP cross-linking method for the photodynamic detection of intestinal neoplasm [128]. In another work, saponin-loaded chitosan–TPP nanoparticles were developed and showed increased toxicity toward cancer cells [129]. In another report, 5-aminosalicylic acid-loaded carboxymethyl chitosan–starch nanoparticles were developed via a complex coacervation process for colon-specific drug delivery [130]. 5-fluorouracil-loaded folate-conjugated manganese-doped zinc sulfide-*O*-carboxymethyl chitosan nanoparticles were reported by our group for targeted drug delivery, with potential usefulness in cancer therapy [2]. From our studies [129], it was confirmed that the system was able to deliver the anticancer drug (5-fluorouracil) along with simultaneous imaging of cancer cells without affecting their metabolic activity and morphology under *in vitro* conditions. In another work, mono-*N*-carboxymethyl chitosan (MCC) and *N*-trimethyl chitosan (TMC) nanoparticles have been developed for non-invasive vaccine delivery [131]. TMC–MCC nanocomplexes have also been used as carriers for the mucosal delivery of vaccines [132].

## 7.5 Conclusions and Future Perspectives

In the current chapter, we provide an overview of various cross-linked as well as chemically modified chitosan derivatives and their processing routes with special consideration to drug delivery applications. In particular, the drug delivery applicability of micro- and nanoparticles of cross-linked as well as chemically modified chitosan derivatives have been discussed. In general, these materials are biocompatible and hemocompatible even after modification, and they possess novel properties such as higher drug-loading efficacy and water solubility. The future scope of these materials can be extended for targeted cancer therapy. The preliminary results from many of the studies on modified chitosan materials strongly support their potential as versatile and effective drug delivery systems and warrant ongoing research in both *in vivo* and preclinical models.

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